UNIVERSITI PUTRA MALAYSIA

POSTPARTUM OVARIAN FUNCTION IN DAIRY CATTLE

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Postpartum ovarian function in dairy cattle

by
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This thesis attached hereto, entitled "Postpartum ovarian function in dairy cattle" prepared and submitted by Laba Mahaputra in partial fulfilment of the requirements for the degree of Master of Science, is hereby accepted.

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ABSTRACT

To achieve the economic target of one calf a year, a cow must be pregnant by 85 days postpartum and is essential for maximum milk production. Resumption of ovarian activity and repro

were investigated in ten normally calving cows from each of three breeds of cattle - Friesian, Jersey x LD and Friesian x Sahiwal. Visual observations, a teaser bull and tail painting wer

detection. Rectal palpation was conducted once weekly between 17 and 70 days postpartum and laparoscopic examination was performed at least once during the experimental period to monitor ovarian changes. Samples of blood plasma were collected twice weekly during the same period from all cows and assayed for plasma progesterone using a radioimmunoassay. The

non-detected oestrus, pregnancy and early embryonic mortality. A plasma progesterone concentration of 1 ng/ml or greater was used as the criterion for luteal activity.

Intervals and standard deviations from parturition to first ovulation and first detected oestrus were 23 $\pm$ 6.5 and 62 $\pm$ 25.9 days for Friesian, 29 $\pm$ 9.9 and 33 $\pm$ 11.5 days for Jersey x LD and 48 $\pm$ 29.1 and 59 $\pm$ 21.9 days for Friesian x Sahiwal cows. Interval from calving to the first postpartum ovulation and oestrus among three breeds were significantly different ($P < 0.05$). The interval from parturition to uterine involution for Friesian, Jersey x LD and Friesian x Sahiwal were 35 $\pm$ 1.2, 33 $\pm$ 4.1 and 30 $\pm$ 2 days respectively ($P > 0.05$). Only 52 percent of all cows were detected in oestrus at the commencement of the first oestrous cycle. The mean plasma progesterone concentration during detected oestrus was 0.23 $\pm$ 0.16 ng/ml and at 21 days for

pregnant cows was 3.01 ± 0.89 ng/ml. Average plasma progesterone levels during oestrous cycle were highest in Friesian cows, moderate in Jersey x LD and lowest in Friesian x Sahiwal cows. Only one case of early embryonic death was recorded. Cystic ovaries were observed in 4 out of 10 Friesian cows. Sixty percent of Jersey x LD and 50 percent of Friesian x Sahiwal had conceived about day 85 postpartum. However, none of the Friesian cows had conceived by this time. The infertility in the Friesian cows were anoestrus, non-detected oestrus and repeat breeding which extended the calving interval beyond the optimum interval. The results confirm that *Bos taurus* x *Bos indicus* dairy breeds have a better reproductive performance than a *Bos taurus* breed in a tropical environment.

INTRODUCTION

In recent years, there has been an increased interest in milk production in Asian countries to meet the demand for animal protein for human consumption. In Malaysia, this demand has been met by importation of dairy cattle (*Bos taurus*) with high milk producing potential from temperate countries. During the past ten years, Malaysia has been importing several breeds of *Bos taurus* cattle, e.g. Friesian and Jersey. Because of the low fertility of *Bos taurus* x *Bos indicus* breed (Friesian x Sahiwal F_1) were imported for milk production. The Malaysian government has imported since 1977, 15,000 to 20,000 head of pregnant F_1 Friesian x Sahiwal cows from New Zealand and Australia, hoping that these crossbred animals would have a much better reproductive performance and adaptability compared to *Bos taurus* breeds in tropical countries such as Malaysia.

A calving interval of approximately 365 days is essential for maximum milk production. A cow must be detected in oestrus by approximately 60 days postpartum and must be pregnant by 85 days if this goal is to be achieved. To identify which of the dairy breeds in Malaysia could maintain a calving interval of 365 days it is necessary to study their postpartum ovarian function.

Ovarian function in cattle has been studied by observation of oestrus behaviour and morphologic ovarian changes occurring in the ovaries which are detectable by rectal palpation, laparotomy or laparoscopy. More recently sensitive radioimmunoassay techniques have been developed for repeated measurement of ovarian and other hormones in biological fluids e.g. blood or milk. Of the ovarian steroids, progesterone concentrations in blood plasma or milk has yielded most

information on ovarian function in cattle, because it reflects very closely the secretory activity of the corpus luteum.

The postpartum period in cattle may be considered as the period from parturition to a fertile oestrus at which the chance of conception is high. The objective of this study was to correlate resumption of ovarian activity, occurrence of first oestrus and temporal patterns of plasma progesterone concentrations over a 70-day postpartum period in one breed of *Bos taurus* (Friesian) and two *Bos taurus* x *Bos indicus* breeds of dairy cattle in Malaysia.

REVIEW OF LITERATURE

The interval between calvings is economically important in dairy herds (Louca and Legates, 1968). Since a herd calving interval of 365-385 days is often regarded as optimal, the average interval from calving (day 0) to conception should be approximately 85 days postpartum. This target can be achieved if the average number of days from calving to first service is about 66 days (Boyd and Reed, 1961; Norman, 1967; Whitmore et al., 1974; King et al., 1976); this interval also allows higher daily production over a series of lactations (Louca and Legates, 1968) and a return over feed cost (Speicher et al., 1967).

Uterine involution

The time for the uterus to return to its non-pregnant state after calving (uterine involution) is important for cows to be bred at the optimum time. Normally, both uterine horns are of the same size by day 25 to 30 postpartum (Morrow et al., 1966; Morrow, 1969), although the cervix is slightly larger than either horn. The uterine mucosal epithelium usually covers the surface of the caruncle in most normal cows by 30 days postpartum (Wagner and Hansel, 1969). Abnormal parturition resulting in endometritis, metritis, pyometra or a retention of placenta delays uterine involution (Morrow et al., 1969) and thereby affect conception.

CL of pregnancy

During pregnancy, the corpus luteum (CL) is the major source of progesterone in cattle (Erb et al., 1968a). The CL of pregnancy always occurred on the ovary adjacent to the postgravid uterine horn (Morrow et al., 1969; Marion & Gier, 1968). In cattle, pregnancy occurs more frequently in the right uterine horn than in the left horn.

Twelve reports (Morrow et al., 1968) showed that the relative activity of the right ovary varied from 50.8 to 67.4 percent. Following parturition CL of pregnancy degenerates by day 7 postpartum. It is approximately 9 mm in diameter by day 14 postpartum and remained at this size, at least to day 30 postpartum (Wagner and Hansel, 1969). The non-functional CL (corpus albicans) does not secrete progesterone (Labhsetwar et al., 1964).

Follicular development

Ovarian follicles are palpable 5 to 7 days following parturition (Morrow et al., 1966; Marion and Gier, 1968; Callahan et al., 1971). At day 7 postpartum, the mean diameter of the largest follicle was 9.6 mm; at day 14, it was 11.33 mm; at day 30, 13.0 mm (Wagner and Hansel, 1969). Interval from parturition to development of ovarian follicles greater than 10 mm is about 15 to 16 days (Saiduddin et al., 1968; Kesler et al., 1979).

Ovulation

The interval from parturition to first ovulation ranges from 17 to 24 days (Britt et al., 1974; Kesler et al., 1978; Saiduddin et al., 1968; King et al., 1976 and Callahan et al., 1971). The first postpartum ovulation occurred earlier in multiparous cows than primiparous cows (Rosenberg et al., 1977). The incidence of ovulation was higher in the ovary opposite the CL of pregnancy before 21 days postpartum than at a later period (Morrow et al., 1968; Marion and Gier, 1968). The incidence of first postpartum ovulation without oestrus behaviour ranged from 15 to 43 percent (Boyd and Munro, 1979; Callahan et al., 1971 and Whitmore et al., 1974). A high level of nutrition could increase the percentage of first ovulation and oestrus as compared to an average nutrition level (Whitmore et al., 1974), but delayed the first postpartum ovulation (Folman et al., 1973). The interval for

the second and third postpartum ovulations were 39.4 days and 58.6 days respectively and the percentage of cows detected in oestrus at the first, second and third ovulation were 19%, 62% and 58% respectively (Sharpe and King, 1981).

Endocrine regulation of postpartum reproductive function

Release of prostaglandin F_2 alfa ($PG_{2\alpha}$) is the key hormonal event in terminating luteal function during late pregnancy in the cow. The regression of the corpus luteum causes an abrupt decrease in the concentration of progesterone in the maternal peripheral blood plasma (Stabenfeldt et al., 1970; Edqvist et al., 1973). The trigger for $PGF_{2\alpha}$ release in late pregnancy is probably a rise in estrogen levels (Challis et al., 1972). The $PGF_{2\alpha}$ levels remain high and do not reach base-line levels until 10-20 days after delivery, however, progesterone and oestrogen levels in maternal blood plasma decrease immediately following parturition (Edqvist et al., 1976; 1978).

The pattern of plasma LH and FSH in cows during the first 5 days postpartum show no clearly defined episodes of either LH or FSH irrespective of whether the cow is milked or suckled. Thereafter, levels of FSH rise first, followed at a variable period by increase in basal levels of plasma LH. This may be related to sporadic endogenous release of GnRH at infrequent intervals giving rise to transient LH release with a return to basal levels of LH but with increased FSH release, causing a more sustained release in plasma FSH levels. Once an increased level of FSH has occurred at an early stage in the post-partum milked cow, differences in plasma FSH are not considered a limiting factor to the onset of ovarian activity (Lamming et al., 1981; Schams et al., 1978).

The development of episodic release of LH may be a prerequisite for the onset of cyclic ovarian activity in the cow (Peters et al.,

1981). The transition to ovarian activity may be due to increased Gn-RH release leading to more frequent plasma LH episodes resulting in ovarian follicular activity and oestradiol secretion which enhances pituitary responsiveness to Gn-RH. In the postpartum milked cow, LH pulses typically appear in plasma at about day 10 but their appearance is delayed in intensively suckling cows which tend to undergo longer postpartum acyclic periods (Carruthers and Hafs, 1980; Peters et al., 1981). In the milked cow, exhibiting ovarian activity by 24 days postpartum, there is a significant rise in basal levels of plasma LH prior to this time and associated with an episodic LH release (Lamming et al., 1981). This is then followed either by a transient rise in milk progesterone (> 3 ng/ml) or plasma progesterone (1-3 ng/ml) for up to 10 days or the normal luteal phase duration (Lamming, 1978; Webb et al., 1980; Lamming et al., 1981). If a transient rise in progesterone occurred then this is followed by a similar preovulatory type of LH surge and
the subsequent oestrous cycle of normal length.

Plasma concentrations of oestradiol-17β declined from pre-partum peaks to basal levels by day 4 to 8 postpartum in suckled and milked cows (Arije et al., 1971; Smith et al., 1973). During the anovulatory phase of the postpartum period large ovarian follicles are present (Kesler et al., 1979) associated with levels of oestradiol-17β as high as those during oestrus (Rawlings et al., 1980). Although estrogen levels are high, majority of otherwise normal cows do not show signs of behavioural oestrus or ovulation during this postpartum anovulatory period.

The regressing CL of pregnancy and plasma draining the ovaries contain very low levels of progesterone, 1 to 4 days postpartum (Labhsetwar et al., 1964). Thereafter, progesterone levels in peripheral blood remain at basal levels for a variable period (Arije et al.,

1971; Robertson, 1972; Lamming et al., 1981; Webb et al., 1980) until the resumption of cyclic ovarian activity. In most cows, the first ovulation is preceded by a short-term elevation of progesterone (Pope et al., 1969; Donaldson et al., 1970; Lamming & Bulman, 1976; Webb et al., 1980). Once normal ovarian activity has been initiated, progesterone secretion by the corpus luteum exhibits a cyclical pattern - periods of low progesterone (follicular phase) alternating with high progesterone (luteal phase).

Assessment of ovarian function

Several clinical and endocrine techniques are available for the study of ovarian function. One of the earliest methods of assessing ovarian activity was the palpation of the ovaries per rectum (Murray, 1959). Rectal palpation is the widely used clinical method of monitoring ovarian activity based on the palpation of ovarian follicles greater than 7 mm in diameter or a CL after day 5 of the oestrous cycle. The recognition of these structures by rectal palpation has been described in detail (Zemjanis et al., 1969). In cattle, the accuracy of rectal palpation for diagnosing a CL ranges from 75 to 90 percent (Dawson, 1975; Boyd and Munro, 1979; Watson and Munro, 1980).

Laparotomy has been employed for direct observation of the ovaries in the live animal but has now been superseded by laparoscopy (Megale, 1956; Wishart & Snowball, 1973; Seeger, 1977; Jainudeen et al., 1982).

The radioimmunoassay (RIA) technique is a highly sensitive, new method of measuring protein and steroid hormones in biological fluids. The basis for the assay consists of the competition between a known amount of labelled hormone and an unknown amount of extracted hormone for binding sites on antibody molecules. The labelled hormone has a high proportion of ^{125}I or ^{3}H atoms in the molecule. After equilibration, the antibody-bound and unbound radioligand are separated and

the radioactivity in the bound form can be determined in a spectrometer and inversely related to the endogenous hormone concentration.

Progesterone levels in peripheral blood or milk measured by RIA exhibits a cyclic variation through the cycle in the cow (Robertson, 1972; Lamming and Bulman, 1976). The progesterone levels have been used for the detection of postpartum acyclicity (Lamming, 1980), the diagnosis of problems associated with oestrus detection and timing of insemination (Appleyard and Cook, 1976; Foote et al., 1980), early identification of non-pregnant cows (Heap et al., 1973; Pope et al., 1976) and differential diagnosis of ovarian dysfunction (Booth, 1980).

Factors affecting resumption of ovarian activity

Resumption of ovarian activity in cattle is regulated by factors such as heat stress, suckling, season, nutrition or weaning. However, the mechanisms by which these factors influence ovarian function are unknown. Plasma or milk progesterone profiles of cows have enabled the study of factors influencing the onset of ovarian cycles and the incidence of various types of subfertility.

Thermal stress results in a variety of reproductive disturbances (Hafez, 1968; Jainudeen, 1976) such as prolongation of the oestrous cycle (Madan and Johnson, 1973), reduction in the intensity of oestrus or anoestrus under severe heat stress (Stott and Williams, 1962; Gangwar et al., 1965; Bond et al., 1972). Heat stress prior to ovulation will block the ovulatory release of LH, thereby changing the temporal pattern of oestrus behaviour and ovulation (Baldwin and Sawyer, 1974). An increased secretion of adrenal corticosteroid hormone (Willet and Erb, 1972) and a decrease in the amplitude of the LH surge prior to ovulation has been reported in cows exposed to high environmental temperatures (Madan and Johnson, 1973).

An inhibitory influence of suckling upon the resumption of ovarian

cycles in cattle has been reported by several investigators. The intensity of mammary stimulation is a major factor in prolonging the postpartum interval (Short et al., 1972; Wetteman et al., 1978). Postpartum anoestrus is more common in suckled than machine-milked cows, beef than dairy cows, cows milked frequently than milked twice daily (Carruthers & Hafs, 1980) and cows suckling twins than single calves (Wetteman et al., 1978). Calf removal hastens the resumption of ovarian cycles in beef cows (Smith et al., 1979; Carter et al., 1980).

Apparently, suckling acts at the hypothalamus and/or pituitary level rather than at the ovarian level. Plasma LH levels are significantly reduced in suckled compared with non-suckled cows (Radford et al., 1978; Carruthers et al., 1980; Walters et al., 1982a) due to a reduction in both the frequency and amplitude of episodic release of LH (Carruthers & Hafs, 1980; Carruthers et al., 1980; Peters et al., 1981). In contrast, weaning calves for 48 or 96 h results in increased basal serum LH concentrations due to increased frequency of pulsatile LH release (Walters et al., 1982b), providing indirect evidence that inhibition of the episodic LH release is due to a reduction in the frequency of Gonadotropic Releasing Hormone (GnRH).

Prolactin does not appear to mediate the effects of suckling on postpartum episodic LH secretion and/or ovulation (Carruthers & Hafs, 1980; Wheeler et al., 1982). Other findings (Li & Wagner, 1980) suggest that hyperadrenal activity may be involved in the mechanism by which suckling suppresses postpartum ovarian function in cattle.

Ovarian disorders

Cystic ovarian disease. This is, by far, the most predominant ovarian pathological condition found in the dairy cow. The incidence of the disease was 22.8 percent of tracts examined in an abattoir

(Al-Dahash and David, 1977) and 18 to 21.5 percent in dairy herds based on a clinical diagnosis (Boyd and Munro, 1979; Kalis, 1980; Karg, 1980). The incidence of cystic ovarian disease is higher in the Holstein-Friesian than in other dairy breeds (Hardie and Ax, 1981) and also in cows which have a higher genetic potential for milk production (Marion and Gier, 1968).

The aetiology of cystic ovarian disease may be due to a deficiency of luteinizing hormone needed to induce ovulation or due to a deficiency of gonadotropin releasing hormone (Erb et al., 1971). Exogenous estrogens, progestins or follicular stimulating hormone may also cause ovarian cysts (Roberts, 1971; Seguin et al., 1976; Kesler et al., 1979).

The diagnosis of cystic ovarian disease is based upon rectal findings of one or more smooth, fluctuant, rounded structures 25 mm in diameter or larger on one or both ovaries (Roberts, 1955; Morrow et al., 1969; Nadaraja & Hansel, 1976; Kalis, 1980). Ovarian cysts are of two types: follicular or luteal cysts. Follicular cysts are thin-walled structures and occur most commonly within 15 to 45 days postpartum. Anestrus is the predominant clinical sign but nymphomania may be observed in cystic cows 60 days postpartum (Morrow et al., 1969). Luteal cysts are Graffian follicles which do not rupture and have a thin rim of luteal tissue. Animals with luteal cysts are anestrus. Although cystic CL occurred following 25 percent of ovulations during the 60-day postpartum period, it is not considered to be of clinical importance because neither the oestrous cycle nor conception is affected (Morrow et al., 1969).

A study comparing clinical diagnosis (rectal palpation) with milk progesterone level (Hoffmann et al., 1976) for the differential diagnosis of follicular and luteal cysts showed that a clinical diagnosis was 65 percent accurate in determining follicular cysts and 80 percent accurate

for luteal cysts when based on the progesterone level in a single sample. However, for clinical purposes, the milk progesterone assay is not generally applicable to differentiate between types of ovarian cysts (Gunzler et al., 1979). Multiple blood or milk samples from cystic cows over an extended period of time have yielded erratic progesterone concentrations (Dobson et al., 1975; Hoffmann et al., 1976; Gunzler et al., 1979).

True anoestrus or inactive ovaries is observed in approximately 12 to 15 percent dairy cows (Boyd and Munro, 1979; Kalis, 1980). A prolonged period of anoestrus is also observed in lactating cows of low body weight (Baker, 1968). In true anoestrus, rectal palpation reveals smaller than normal ovaries which are smooth and often fibrotic (Roberts, 1971) associated with plasma progesterone levels less than 0.5 ng/ml (Robertson, 1972).

Persistence of the corpus luteum. Anoestrus associated with prolonged luteal function
such as pyometra, or mummified foetus (Roberts, 1971). However, plasma and milk progesterone profiles have revealed a prolongation of the luteal phase beyond the normal 15-day life span of the CL in postpartum cows which had not been inseminated (Lamming and Bulman, 1976).

Short luteal phase. During the postpartum period, the first oestrous cycle in 52 percent dairy cows was shorter than normal (Schams et al., 1978). Although the secretory activity of the CL following the first ovulation as determined by progesterone levels in plasma or milk is normal, the life span of the CL is shorter than during a normal oestrous cycle (Marion & Gier, 1968; Morrow et al., 1966).

MATERIALS AND METHODS

Experimental animals

Thirty dairy cows belonging to the Universiti Pertanian herd, calving normally between August 1981 study. The experimental animals were 10 Friesian, 10 F_1 Jersey x Local Dairy (J x LD), and 10 Friesian x Sahiwal (F x S) crossbreds.

The Friesian (F) animals were daughters of cows imported from Australia and the F_1 crosses were the daughters of Local Dairy cows inseminated with imported Jersey semen and Friesian cows inseminated with Sahiwal semen in New Zealand. Most F and J x LD cows were multiparous whereas F x S cows were all primiparous. Friesian cows were housed and fed cut fodder and the crossbreds were maintained in paddocks of established pastures consisting of Panicum maximum (Guinea grass), Setaria anceps (Setaria nandi), Pennisetum purpureum (Napier grass) and Cynodonplecostochyus (African star grass). All animals had free access to water and mineral licks containing 10 percent phosphorus in the form of monosodium phosphate in 50 percent table salt and recommended trace elements.

All cows were machine milked twice daily at 05:00 and 16:00 h and were fed a concentrate mixture containing 15 percent protein and 3.67 Mcal ME/kg at the rate of 3 to 4 kg per animal at each milking.

Rectal palpation

At weekly intervals (day 17 to 70 postpartum) the reproductive organs of all cows were examined rectally. The position of the uterus in the body cavity and the diameter at the external bifurcation of each horn was recorded. Uterine involution was complete when both uterine

horns reached normal nongravid size and position. The ovaries were palpated to estimate size and to record palpable structures such as follicles (F) and corpus luteum (CL). The day of ovulation was estimated to be 4 days before the time a CL was first palpable i.e., approximately one-half the time since the previous palpation.

Oestrus detection and insemination

Cows were penned at 20:00 h and tested for oestrus using a vasectomized bull fitted with a chin-ball marking device, tail painting (Macmillan and Currow, 1977) and visual observation. The male remained with the cows until the next morning. A cow was considered as in oestrus (day 0) if it stood to be mounted, marked by the bull or had its tail paint removed. All animals were artificially inseminated by an experienced inseminator approximately 12 hours aft
oestrus which occurred between 50 to 60 days postpartum. Friesian or Jersey frozen semen of known fertility in 0.25 ml French straws was deposited in the uterine body. Cows not returning to oestrus were rectally examined for pregnancy 45 to 60 days after insemination.

Laparoscopy

Cows were subjected to laparoscopic observation of the ovaries between day 30 to 70 postpartum using the technique developed for the buffalo (Jainudeen *et al.*, 1982).

Equipment. The equipment for lapar
Germany) consisted of: an electronic flash generator containing a cold-light source from a Halogen lamp and proximal photoflash source (# 5006), a 0^{o} vision Lumina telescope (10 mm diameter, 600 mm length), a special light transmitting cable (4.5 mm diameter, 180 mm length), a trocar (11 mm diameter) with a pyramidal tip and a corresponding trocar sleeve with a piston valve (145 mm working length), an accessory trocar-sleeve unit (6 mm diameter) for the passage of a manipulating blunt-ended steel

probe (5 mm diameter, 750 mm length), and a CO_2 cylinder fitted with a pressure reducing valve and a flowmeter. All laparoscopic equipment were steam-sterilized - except the laparoscope and light transmitting cable. The laparoscope was immersed in a germicidal solution (Savlon, ICI) for 30 to 60 minutes before use.

The photographic system consisted of a 35 mm camera body (Olympus) and a 95 mm lens (RIWO) which were mounted on the eyepiece of the 10 mm laparoscope. The camera shutter was synchronized with the flash generator (5006, Wolfe). Laparoscopic findings were documented on colour positive film (ASA 200, Kodak High Speed Ektachrome Daylight Film) at shutter speeds of 1/15, 1/30 and 1/60 sec.

Laparoscopic procedure. Feed and water were withheld from cows for 24 to 48 hours prior to laparoscopy.

All laparoscopic examinations were conducted on cows standing portable crush 10 min. after an intravenous injection of a 2% Xylazine (Rompun, Bayer) solution at a dose rate of 4 mg/100 kg body weight. The animal was positioned with hindquarters elevated. Two sites (5 x 5 cm) in the upper and lower thirds of the right paralumbar fossa were shaved, cleaned and disinfected and infiltrated with 20 ml of a 4% solution of procaine hydrochloride solution to desensitize skin, muscles and peritoneum. Two vertical incisions, 10 to 15 mm long, were made through skin and muscles.

A 11 cm trocar-sleeve assembly was inserted through the lower incision in a caudal and slightly ventral direction into the abdominal cavity. The laparoscope was inserted through the sleeve into the abdominal cavity. At this stage, the abdominal cavity was insufflated with CO_2 (5 l/min). Next, the manipulating probe was inserted through the upper incision via the 6 mm trocar-sleeve assembly to probe the ovarian surfaces and adnexa.

After ovarian structures (follicles, corpus luteum) were photographed, the manipulating rod, the laparoscope and their sleeves were withdrawn, followed by closure of the skin incisions with a single suture of catgut.

PROGESTERONE RADIOIMMUNOASSAY

Collection of samples

Jugular blood samples were collected into evacuated heparinized tubes from each animal at 8:00 h on Mondays and Thursdays between days 17 and 70 postpartum. Samples were also collected on day o insemination and 21 to 24 days later. The plasma was separated immediately by centrifugation at 3000 rpm for 15 minutes, and stored at $-20^{o}C$ in a freezer pending analysis.

Chemicals and apparatus

Sodium azide was laboratory reagent grade from Sigma Chemical Co., St. Louis, USA and gelatin (granular powder, laboratory reagent) was from UCB, Brussels, Belgium. The charcoal suspension was prepared from Norit A charcoal and dextran T-70, both obtained from Sigma Chemical Co. Progesterone was purchased from Sigma Chemical Co., (1, 2, 6, 7-^{3}H) progesterone (87 Ci/mmol) from Amersham International Ltd., Amersham, UK). Scintillation fluid was prepared from toluene (May & Baker, England) and 2, 5-diphenyloxazole (PPO) Sigma Chemical Co., USA. Petroleum ether (Analar) with a boiling point from 40 to $60^{o}C$ was purchased from BDH Chemicals Ltd., Poole, England.

Pipetting was carried with "Pipetman" (Gilson, France) or "Quickpette" (Helen Lab., Texas, USA). Glass tubes were used for extraction (12 x 100 mm) and the assay (10 x 75 mm). Glass scintillation vials (20 ml) were used for counting.

The equipment used included a supermixer (Lab Line Instruments,

Inc., Melrose Park, Illinois, USA), multitube vortexer (Scientific Manufacturing Industries, USA, model 2601), a refrigerated centrifuge (International Equipment Co., USA), a liquid scintillation β-counter (Packard Instruments, USA, model C-300) and a programmable calculator (T-59, Texas Instruments, USA).

Preparation of reagents

Phosphate-buffered saline (PBS) containing 0.1 M sodium phosphate (pH 7.0) with 0.9% NaCl and 0.1% sodium azide (Abraham et al., 1977) was prepared as follows:-

To a 2-litre volumetric flask was added 32.7 g sodium phosphate dibasic heptahydrate (MW 268), 10.8 g sodium phosphate monobasic monohydrate (MW 138), 2.0 g sodium azide (MW 65) and 18.0 g sodium chloride (MW 58). Deionized water was added up to a total volume of 2 litres. The assay buffer also contained 0.1% gelatin (PBSG). The buffer was stored at $4^{o}C$.

Radioactive and non-radioactive progesterone were diluted with ethanol and stored in a freezer ($-20^{o}C$). When labelled progesterone was used as tracer in the RIA, it was evaporated to dryness in a flask and PBSG was added to yield approximately 20,000 dpm ^{3}H-labelled progesterone in 100 µl of assay buffer.

To prepare dextran-coated charcoal suspension, 625 mg Norit A and 62.5 mg dextran T-70 were added to 100 ml of assay buffer, mixed on a magnetic stirrer and stored at $4^{o}C$ in a refrigerator until used. When in use the suspension was kept on a stirrer in order to obtain a homogenous charcoal-buffer solution.

The scintillation fluid contained 5 g PPO per litre of toluene. Quality control (QC) samples were two pools of buffalo plasma containing 0.9 ng/ml (low) and 1.5 ng/ml(high) concentration of progesterone. Aliquots from each pool were stored at $-20^{o}C$ in small amounts sufficient